Monday 5th August

Gel of PCR 3

1. Preparation of gel as previous.
2. Quantities of each component put into gel.

Eppendorf	DNA	Dye	TAE buffer
	Volume of component (µl)		
1 (norV 0.1)	3	3.5	13.5
2 (norV 1)	3	3.5	13.5
3 (NrfA 0.1)	3	3.5	13.5
4 (NrfA 1)	3	3.5	13.5
5 (norV 0.1)	3	3.5	13.5
6 (norV 1)	3	3.5	13.5
7 (NrfA 0.1)	3	3.5	13.5
8 (NrfA 1)	3	3.5	13.5
9(Ladder)	3	3.5	13.5

3. PCR non successful, no bands present on the gel. Conclusion, run next PCR with taq polymerase and not PWO proofreading polymerase.

PCR of purified norV and whole cell NrfA

1. Preparation of primer working stock solution - 2µl of primer and 18µl of water.
2. Suspension of 2 separate colonies into 50µl of water, to act as NrfA template.

Eppendorf	Buffer	dNTP's	Primer-F	Primer-R	DNA template	Taq polymerase	Water
	Volume of component in each eppendorf (µl)						
1	5	5	1.5	1.5	0.1	0.5	36.4
2	5	5	1.5	1.5	1	0.5	35.5
3	5	5	1.5	1.5	0.1	0.5	36.4
4	5	5	1.5	1.5	1	0.5	35.5
5	5	5	1.5	1.5	0.1	0.5	36.4
6	5	5	1.5	1.5	1	0.5	35.5
7	5	5	1.5	1.5	0.1	0.5	36.4
8	5	5	1.5	1.5	1	0.5	35.5

Section of program	Time (minutes)	Temperature (°C)
Initial	15	95
Main cycle 39x		
Initial denaturation	0.5	94
Annealing	0.5	50
Extension	3.5	72

Final extension	20	72

Preparation of 100ml SOC media

- 2.0g of tryptone
- 0.5g of yeast extract
- 19mg of KCl
- 50mg NaCl
- 100ml of distilled water
- 95.2mg of $MgCl_2$
- 24.0mg of $MgSO_4$

1. Weight out masses of solids.
2. Addition of solids to an appropriately sized glass jar.
3. Addition of distilled water.
4. Seal and autoclave.

Transformation of GFP into top 10 competent cells

1. Competent cells on ice. Label 2.0ml centrifuge tubes with GFP 1 and 2.

2. 2µl of miniprep product of GFP should be pipetted into its corresponding tube. A different pipette tip should be used for each.

3. 50µl of competent cells should then be pipetted into each tube. Once this is added, flick each tube, to ensure that they are mixed.

4. Incubate on ice for 30 minutes.

5. Place cells into a 42°C water back, for 1 minute, to heat-shock the cells. To allow the cells to recover, transfer them back to ice and leave them for 5 minutes.

6. Add 200ul of SOC media per tube, and incubate at 37°C for 2 hours. Label agar plates with GFP sample that is to be added.

7. In turn pipet 70ul from each tube onto the appropriate plate, and spread the mixture evenly across the plate. When doing this make sure that the spreading rod is constantly sterile, using ethanol and Bunsen flame. Cool rod on the agar, before spreading cells across plate. Once complete, tape plates in stacks and place in 37°C oven for incubation overnight.

Note: 2 plates from tube 1 and 1 plate was made from tube 2.

Thursday 8th August

Purification of PCR product –norV and linearised plasmid

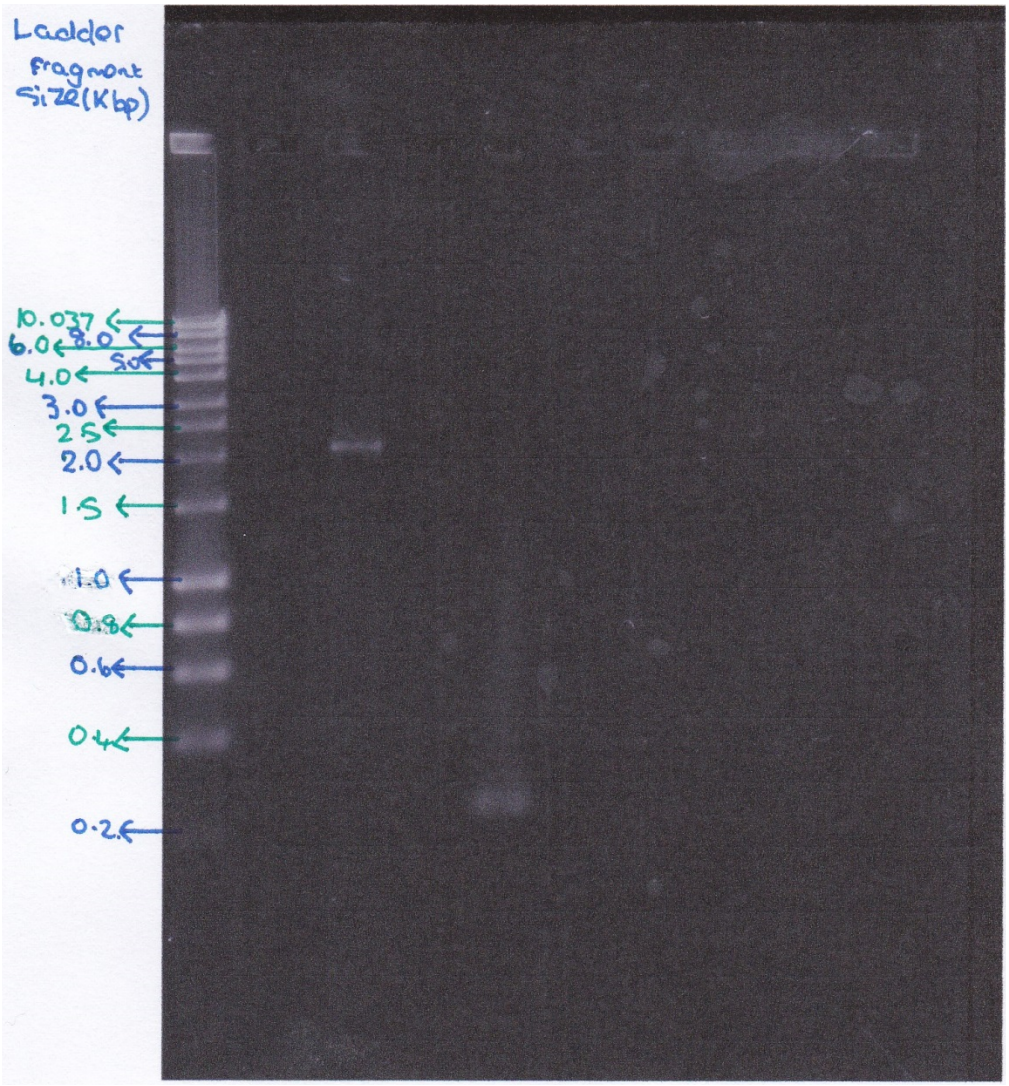
1. Addition of 30µl of binding buffer to each PCR product (1:1 ratio).
2. Addition of isopropanol to norV PCR product. Due to small size of the DNA.

3. Transfer each to GeneJET purification column, centrifuge for 60 seconds at 12,000 rpm. Discard flow through.
4. Addition of 700µl of wash buffer. Centrifuge for 60 seconds at 12,000 rpm. Discard flow through.
5. Recentrifuge GeneJET purification column for 60 second at 12,000 rpm. Discard flow through. Put column into 1.5ml Eppendorf.
6. Add 50µl of elution buffer to tube. Centrifuge for 60 seconds at 12,000 rpm. Keep product at -20ºC.

Agarose gel of purified norV and linearised plasmid

Eppendorf	DNA	Dye	TAE buffer
	Volume of component (µl)		
1 (Ladder)	3	3.5	13.5
2 (Plasmid)	3	3.5	13.5
3 (norV)	3	3.5	13.5

Ladder: lane 2 Plasmid: lane 4 norV: lane 6



Concentration of each purification can be worked out from the gel, by comparing the known concentration of the band in the ladder nearest to the band of the sample in the gel.

Note: take into consideration the volume of ladder added and the volume of DNA. In order to get the concentration of purified DNA in $ngμl^{-1}$. Can be done using software to compare brightness of band of the closest size or just compare by ratio of the mass of the band in the ladder to required band.

Digestion of GFP plasmid

23μl of GFP plasmid,
2μl of Xba1
2μl of Pst1
3μl of 10x buffer H (Roche)
- Above quantities of each into Eppendorf overnight.

Friday 9th August

Digest for control of GFP digest

Eppendorf	Restriction enzyme	Buffer H	Distilled water	GFP miniprep DNA
	Volume of component (μl)			
1 (None)	0.0	0.8	4.2	3
2 (Xba1)	0.2	0.8	4.0	3
3 (Pst1)	0.2	0.8	4.0	3
4 (Both)	0.2 of each	0.8	3.8	3

Put into 37ºC incubator for 2.5 hours.

Ligation of linearised plasmid and norV

- 5μl of plasmid added to each ligation mix – 20ng of DNA.
- 1μl of ligase and 1μl of 10x ligase buffer required for each ligation mix.
- Make 5x dilution of insert to volume of 10μl (2μl of insert and 8μl of water). This can be used in the 3:1 and 1:1 plasmid to insert ligation mixes.

Ratio of plasmid to insert	Mass of insert added (ng)	Volume of insert added (μl)	Distilled water (μl)
3:1	0.83	0.5 (5x)	2.5
1:1	2.5	1.6 (5x)	1.4
1:3	7.5	0.94	2.06
1:0	0	0	3

- Ligation mixes left on the bench over the weekend.